

May 22, 1890.

Sir G. GABRIEL STOKES, Bart., President, in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read :—

I. "A Contribution to the Etiology of Diphtheria."\* By  
E. KLEIN, M.D., F.R.S. Received April 25, 1890.

The microbe, which was first described by Klebs (at the Wiesbaden Congress in 1883), then isolated and grown in artificial cultures by Löffler ('Mitth. aus dem K. Gesundheitsamte,' vol. 2) from human diphtheritic membrane, was shown by this observer to act virulently on various animals. The Klebs-Löffler bacillus—by which name the diphtheria microbe is known—is the one with which also Roux and Yersin ('Annales de l'Institut Pasteur,' vol. 2, 1888, No. 12) obtained positive results on guinea-pigs.

In the Reports of the Medical Officer of the Local Government Board for 1888–1889 and 1889–1890, I have shown that there occur in diphtheritic membranes two species of bacilli, very similar in morphological respects, and also in cultures on serum and on agar, but differing from one another in this, that one species, Klebs-Löffler bacillus No. 1, is not constant in diphtheritic membranes, does not grow on solid gelatine at 19–20° C., and does not act pathogenically on animals; the other species, Klebs-Löffler bacillus No. 2, is constant in diphtheritic membranes, in fact is present even in the deeper layers of the membranes in great masses and almost in pure culture, acts very virulently on animals, and grows well on gelatine at 19–20° C. Löffler, and after him other observers (Flügge, 'Die Mikroorganismen,' 1886), considered it as a character of the diphtheria bacillus that it does not grow on gelatine below 22° C., but this character, though true of the Klebs-Löffler species No. 1, does not appertain to the diphtheria bacillus species No. 2. In fact, there is no difficulty in obtaining pure cultures of this bacillus on gelatine if a particle of diphtheritic membrane be taken and well shaken in two or three successive lots of sterile salt solution, and from the last lot plate

\* This research was undertaken for the Medical Department of the Local Government Board, and is communicated to the Royal Society with the permission of the Medical Officer.

cultivations on gelatine are made. In this way I have obtained the diphtheria bacillus in great numbers of colonies and in pure culture. Zarniko ('Centralbl. f. Bakteriöl. u. Parasit.,' vol. 6, 1889, p. 154) and Escherich (*ibid.*, vol. 7, 1890, p. 8) both state that the diphtheria bacillus does grow on gelatine below 20° C.

This bacillus diphtheriæ acts very virulently on guinea-pigs on subcutaneous inoculation; at the seat of the injection a tumour is produced, which in its pathology and in microscopic sections completely resembles the diphtheritic tissue in man. In human diphtheria the diphtheria bacillus is present only in the diphtheritic membrane, but neither in the blood nor in the diseased viscera; the same holds good for the experimental guinea-pigs. In subcutaneous inoculation with artificial culture, though it causes in these animals acute disease and death—the lungs, intestine, and kidney are greatly congested—the diphtheria bacillus remains limited to the seat of inoculation. It was for these reasons that Löffler concluded that in diphtheria the diphtheritic membrane alone is the seat of the multiplication of the diphtheria bacillus, and that here a chemical poison is produced, which absorbed into the system causes the general diseased condition and eventually death. Roux and Yersin have then separated from artificial broth cultures the bacilli and the chemical products, and, by the injection of these latter alone into guinea-pigs, have produced a general effect. I have in this year's Report to the Medical Officer of the Local Government Board (1889–1890) shown that in these experiments of injection of cultures into guinea-pigs, an active multiplication of the diphtheria bacilli at the seat of inoculation can be demonstrated by culture experiments; from the local diphtheritic tumour and the nearest lymph glands the diphtheria bacilli can be obtained in pure culture on gelatine.

On various occasions during the last three years information has reached me by Health Officers (Dr. Downes, Mr. Shirley Murphy, Dr. Thursfield) as to a curious relation existing between a mysterious cat disease and human diphtheria in this manner, that a cat or cats were taken ill with a pulmonary disease, and while ill were nursed by children, and then these latter sickened with well-marked diphtheria. Or children were taken ill with diphtheria, and either at the same time or afterwards the cat or cats sickened. The disease in the cat was described as an acute lung trouble; the animals were quiet, did not feed, and seemed not to be able to swallow; in some cases they recovered, in others they became emaciated, while the lung trouble increased, and ultimately they died. In one instance—in the north of London, in the spring, 1889—this cat malady, occurring in a house where diphtheria soon afterwards appeared amongst the children, was of a widespread nature; a veterinary surgeon—Mr. Daniel—informed me that at that time he had several

patients amongst cats affected with the disease, consisting in an acute catarrhal affection, chiefly of the respiratory passages. He furnished me with two such animals: one that after an illness of several weeks had died, another that was sent to me in a highly emaciated state, affected with severe broncho-pneumonia; this animal was paralysed on the hind limbs. In both instances the *post-mortem* examination showed severe lung disease, broncho-pneumonia, and large white kidneys due to fatty degeneration of the entire cortex. A similar condition is met with in the human subject in diphtheria. Further, I received from Dr. Thursfield, of Shrewsbury, the body of a cat that had died after a few days' illness from pneumonia in a house in which children were ill with diphtheria; another cat in the same house that became next ill with the same lung trouble also succumbed. The *post-mortem* examination of the animal that I received showed severe broncho-pneumonia and large white kidneys, the entire cortex being in a state of fatty degeneration.

Subcutaneous inoculations of cats were carried out with particles of fresh human diphtheritic membranes and with cultures of the diphtheria bacillus ('Report of the Medical Officer of the Local Government Board,' 1889-1890); hereby a local diphtheritic tumour was produced at the seat of inoculation, and a general visceral disease; in the cases in which death followed after a few days the lungs were found much congested; when death followed after one or more weeks, the lungs showed broncho-pneumonia and the kidneys were enlarged and white, the cortex being in a state of fatty degeneration; if the disease in the animals lasted beyond five to seven days, both kidneys were found uniformly white in the cortex; if of shorter duration, the fatty degeneration was sometimes only in patches. Although in these experiments the bacillus diphtheriæ was recoverable by cultivation from the diphtheritic tumour at the seat of inoculation, there were no bacilli found in the lungs, heart's blood, or kidney, and the conclusion is justified that, just as in the human diphtheria and in the diphtheria produced by subcutaneous inoculation in the guinea-pig, so also in these experimental cats the visceral disease must be a result of the action of a chemical poison produced by the diphtheria bacillus at the seat of inoculation.

From this it is seen that the similarity between the artificial disease and the natural disease in the cat is very great, and the question that presents itself is, In what manner does the animal receive or give the diphtheritic contagium in the natural disease? The natural disease in the cat is in its symptoms and pathology a lung disease, and it is reasonable to suppose from analogy that the lung is the organ in which the diphtheritic process in the cat has its seat. The microscopic examination of the diseased lung of cats that died from the natural disease bears this out, the membrane lining the bronchi in the

diseased portions of the lobules presenting appearances which in microscopic character coincide with the appearances in the mucous membrane of the human fauces, pharynx, or larynx in diphtheria. But the correctness of the above supposition, that diphtheria has its seat in the lung of the cat naturally diseased, was proved by direct experiment. Broth culture of the bacillus diphtheriæ was introduced into the cavity of the normal trachea without injuring the mucous membrane. The animals became ill with acute pneumonia, and on *post-mortem*, two to seven days after, there was found extensive pneumonia, and fatty degeneration of the kidney. The bronchi, infundibula, and air cells of the inflamed lobules were found occluded by, and filled with, exudation which under the microscope bears a striking resemblance to human diphtheritic membranes, and in the muco-purulent exudation in the large bronchi and trachea the diphtheria bacilli were present in large numbers.

During the last ten or twelve years certain epidemics of diphtheria have occurred which were traced to milk, but the manner in which that milk had become contaminated with the diphtheritic virus could not be demonstrated, although the evidence as to the milk not having been directly polluted from a human diphtheria case was very strong. The epidemic of diphtheria that prevailed in the north of London, in 1878, investigated by Mr. Power for the Local Government Board, then the epidemic that occurred in October, 1886, at York Town and Camberley, the epidemic in Enfield, at the beginning of 1888, and in Barking, towards the autumn of 1888, were epidemics of this character. Mr. Power, in his Report to the Local Government Board on the York Town and Camberley outbreak, states (page 13) that a veterinary surgeon had certified that the cows from which the infected milk was derived were all in good health, but that two of the cows showed "chaps" on their teats, and he adds that even two or three weeks after the epidemic had come to an end—the use of milk having been in the meanwhile discontinued—he saw at the farm one cow which had suffered from chapped teats. At Enfield a veterinary inspector had also certified that the cows were in good health; but at Barking the veterinary inspector found sores and crusts on the udder and teats of the cows.

I have made experiments on milch cows with the diphtheria bacillus, which appear to me to throw a good deal of light on the above outbreaks of diphtheria.

Two milch cows\* were inoculated with a broth culture of the diphtheria bacillus derived from human diphtheria. In each case a Pravaz syringe-ful was injected into the subcutaneous and muscular

\* The cows had been kept under observation previous to the experiment for ten days and were in all respects perfectly normal.

tissue of the left shoulder. On the second and third days there was already noticed a soft but tender swelling in the muscle and the subcutaneous tissue of the left shoulder; this swelling increased from day to day, and reached its maximum about the end of the week; then it gradually became smaller but firm. The temperature of both animals was raised on the second and third day, on which days they left off feeding, but after this became apparently normal. Both animals exhibited a slight cough, beginning with the eighth to tenth day, and this gradually increased. One animal left off feeding and ruminating on the twelfth day, "fell in" considerably, and died in the night from the fourteenth to fifteenth day; the other animal on the twenty-third to twenty-fourth left off taking food, "fell in" very much, and was very ill; it was killed on the twenty-fifth day.

In both animals, beginning with the fifth day, there appeared on the skin of the udder, less on the teats, red raised papules, which in a day changed into vesicles, surrounded by a rim of injected skin. The contents of the vesicles were a clear lymph; the skin underneath was much indurated and felt like a nodule; next day the contents of the vesicle had become purulent, *i.e.*, the vesicle had changed into a pustule; in another day the pustule dried into a brownish-black crust, with a sore underneath; this crust became thicker and larger for a couple of days, then became loose, and soon fell off, a dry healing sore remaining underneath. The whole period of the eruption of papules, leading to vesicles, then to pustules, and then to black crusts which, when falling off, left a healing dry sore behind, occupied from five to seven days. The eruption did not appear in one crop: new papules and vesicles came up on the udder of one cow almost daily between the fifth and eleventh day after inoculation, in the other cow between the sixth and tenth day; the total number of vesicles in the former cow amounted to about 24 on the udder, four on the teats; in the latter they were all on the udder and amounted to eight in all. The size of the vesicles and pustules differed: some were not more than  $\frac{1}{8}$ th of an inch, others larger, up to  $\frac{1}{2}$ — $\frac{3}{4}$  of an inch in diameter; they had all a rounded outline, some showed a dark centre. From one of the above cows on the fifth day milk was received from a healthy teat, having previously thoroughly disinfected the outside of the teat and the milker's hand; from this milk cultivations were made, and it was found that 32 colonies of the diphtheria bacillus without any contamination were obtained from 1 cubic centimetre of the milk.

Unlike in man, in the guinea-pig and in the cat the diphtheria bacillus passed from the seat of inoculation into the system of the cow; this was proved by the demonstration of the diphtheria bacillus in the milk. But also in the eruption on the udder, the presence of the diphtheria bacillus was demonstrated by microscopic

specimens and particularly by experiment. With matter taken from the eruption—vesicles and pustules—of the udder, two calves were inoculated into the skin of the groin; here the same eruption made its appearance: red papules, rapidly becoming vesicular, then pustular, and then becoming covered with brown-black crusts, which two or three days after became loose and left a dry healing sore behind. More than that, the calves that showed this eruption after inoculation became affected with severe broncho-pneumonia and with fatty degeneration of the cortex of the kidney. In the two cows above mentioned, on *post-mortem* examination, both lungs were found highly congested, œdematous, some lobules almost solid with broncho-pneumonia in the upper lobes and the upper portion of the middle or lower lobe respectively; the pleural lymphatics were filled with serum and blood. Hæmorrhages in the pericardium and lymph glands, and necrotic patches were present in the liver. At the seat of inoculation there was in both cases a firm tumour consisting in necrotic diphtheritic change of the muscular and subcutaneous tissue. In this diphtheritic tumour continuous masses of the diphtheria bacillus were present; their gradual growth into and destruction of the muscular fibres could be traced very clearly.

It appears then from these observations that a definite disease can be produced in the cow by the diphtheria bacillus, consisting of a diphtheritic tumour at the seat of inoculation with copious multiplication of the diphtheria bacillus, a severe pneumonia, and necrotic change in the liver; the contagious nature of the vesicular eruption on the udder and excretion of the diphtheria bacillus in the milk prove that in the cow the bacillus is absorbed as such into the system.

From the diphtheritic tumour by cultivation, pure cultures of the diphtheria bacillus were obtained; a small part removed from the tumour with the point of a platinum wire, and rubbed over the surface of nutrient gelatine or nutrient agar, yielded innumerable colonies of the diphtheria bacillus without any contamination. In cultural characters in plate, streak, and stab cultures, and in cover-glass specimens of such cultures, this cow diphtheria bacillus coincided completely with the human diphtheria bacillus, but in sections through the diphtheritic tumour of the cow a remarkable difference was noticed between it and the bacillus from the cultures; inasmuch as in the tissue of the tumour the masses of the microbe, both in the necrotic parts, as also where growing into and destroying the muscular fibres, were made up of filaments and granular threads. But that it was really the diphtheria bacillus was proved by culture experiments and by cover-glass specimens. In the latter, the transitional forms between typical diphtheria bacillus and long filaments with terminal knob-like swellings, with spherical or oblong granules

interspersed here and there in the threads, could be easily ascertained. In the large number of cultivations that were made of the fresh tumour in both cows, the colonies obtained were all of one and the same kind, viz., those of the diphtheria bacillus; no contamination was present in any of the cultivations.

### Appendix. May 20.

Since the above was sent in, the following instructive observations were made with regard to diphtheria in the cat and cow:—

At the beginning of the month of April two cats died at the Brown Institution which had been ill for several days previously. Their illness bore a remarkable similarity with the illness mentioned in the preceding pages as the natural (diphtheritic) disease of the cat, the most prominent symptom being an acute catarrhal affection of the respiratory tract; the animals became much emaciated and died. These two cats—which I will call Nos. 1 and 2—had been quite well previously and were kept in cages in a special shed, in which normal cats are generally kept and used for laboratory purposes. Now, after the above two cats, all cats which were put into this shed became affected in the same way: running of nose and eye, injected conjunctiva with muco-purulent discharge from the eye: coughing and more or less severe bronchial catarrh; the animals were quiet and did not feed. Between the beginning of April and the beginning of May fourteen cats became so affected, some more intensely than others; of these several apparently recovered after an illness of about one week to a fortnight, while five became greatly emaciated, very weak, and the lung trouble having greatly increased, they died, the illness lasting two to three weeks. In all five animals the lungs showed distinct signs of lobular pneumonia. In one cat (which I will call No. 3) there was present in the lower part of the larynx and the upper part of the trachea a whitish false membrane indistinguishable from human diphtheria membrane; sections through these parts conclusively prove this. In a second cat (No. 4) the trachea and bronchi contained a thick layer of fibrinous and purulent matter; in the other three animals the bronchi and infundibula contained purulent fibrinous exudation, but the trachea did not show any naked-eye change. In all five animals both kidneys were found conspicuously enlarged and white, the entire cortex being in a state of fatty degeneration. It is clear from this that the disease in these animals was the same disease as was mentioned above as the natural as well as the artificially induced diphtheria. Further confirmation was obtained by microscopic examination of the diphtheritic larynx and trachea of cat 3. On sections made through the affected portion of the larynx and trachea the entire mucous membrane was found con-

verted into a swollen infiltrated tissue undergoing necrosis; in this tissue the typical diphtheria bacilli could be seen in large and small nests and groups in the superficial layers, and extending from them into the deeper portions of the necrotic membrane. Also in cat 4 cover-glass specimens of the tracheal and bronchial exudation showed the presence of the typical diphtheria bacilli. Now, the above epidemic started with the disease of the two cats, 1 and 2, about the end of March, and the question arises: how did the disease originate in these two animals? No cats had been ill in this shed, and the two cats were normal when some weeks previously they were received at the Brown Institution. But during the latter half of March I had two milch cows in the stables of the Brown Institution ill with diphtheria induced by inoculation with the bacillus from human diphtheria, in fact the two cows described on a former page. They were inoculated on March 17, and, as was mentioned on a former page, showed the peculiar eruption on the udder between the 5th and 11th day; in one animal on the 5th day, *i.e.*, March 21, the diphtheria bacillus was demonstrated in the milk drawn from the udder. As soon as the eruption on the udder and the pulmonary affection in the cows were noticed strict orders were given to the attendant that the milk of both cows was to be thrown away. This order was not obeyed, since part of the milk was given to the two cats above mentioned, and these two animals became affected during the last week of March. I ought to mention, however, that, though the time at which these cats became affected is in perfect harmony with the suggestion that the consumption of the above milk of the affected cow had been the cause of their illness, the man who attended to the cows was also attending to the cats. But in view of the fact that this person was free from diphtheria, the possibility of having conveyed the disease from the cow to the cat is out of the question, particularly if we remember that milk containing the diphtheria bacilli had been actually given to the cats.

II. "The Chemical Products of the Growth of *Bacillus anthracis* and their Physiological Action." By SIDNEY MARTIN, M.D., Pathologist to the Middlesex Hospital. Communicated by Dr. KLEIN, F.R.S. Received May 7, 1890.

The work here recorded was done for the Medical Officer of the Local Government Board, whose permission I have for publishing this abstract of it.

The research was commenced in May, 1889. The bacilli were grown in a solution of pure alkali-albumin (made from serum-